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EFFECT OF DIFFERENT FERMENTATION DURATION AND DRYING METHODS ON THE QUALITY OF COCOA BEAN OIL

BY

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A THESIS SUBMITTED TO THE SCHOOL OF RESEARCH AND GRADUATE STUDIES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE MASTER OF PHILISOPHY (M.Phil.) IN POSTHARVEST TECHNOLOGY.

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DECLARATION

I, Bernard Toku-Appiah hereby declare that this work herein submitted, as thesis is the results of my own investigations. References made therein are however respectively acknowledged.

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DEDICATION

This work is dedicated to my dear wife, Mrs. Evelyn Toku-Appiah and my parents Mr. Kwame Toku-Appiah and Mrs. Agnes Toku-Appiah for the encouragement and support they gave me when I took the decision to undertake a graduate programme.

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ABSTRACT

A study was carried out to assess the quality of oil from cocoa seeds (*Theobroma cacoa*). Fully riped cocoa pods were harvested and the seeds (beans) were fermented for 5-days and 7-days fermentation durations, using 10kg of cocoa beans for each treatment. The fermented beans were afterwards dried by the sun and solar drying methods, under a 2 by 2 factorial in a completely randomized design. Six hundred grams of the dried cocoa beans were milled using a hammer mill and sieved with a 1.18mm standard sieve to obtain fine particle size. Using the Soxhlet's extraction procedure, the oil was extracted. Afterwards, the yield and other quality parameters including acid value, relative density, saponification value, peroxide value, iodine value, refractive index and free fatty acid content, were assessed. The oil yield range (24.10-29.00%) for the interactive effect was low, compared to oil rich seeds used as commercial sources of oil. The study revealed that, though the cocoa bean oil produced from both 5-day and 7-day fermentation durations all had acid values and free fatty acid percentages with significant differences between them but they were all within the range for consumption (0-10). On the other hand, oil produced from 7-day fermentation duration of cocoa beans had the least peroxide value. Though the acid values and free fatty acid contents of the oils produced from the different drying methods were numerically different between the two drying methods, they were not significantly different. Furthermore, the acid values and free fatty acid contents were all within the acceptable range for consumption From the interactive effects, it was also determined that 7-day fermentation duration and solar drying produced the best quality oil among all the interactive effects.

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CHAPTER ONE

1.0 INTRODUCTION

Cocoa, which is processed into cocoa butter, is a very important raw material for the chocolate industry. Cocoa beans for sale are obtained from the fermentation and drying of seeds from the ripe pods of the cocoa plant, *Theobroma cacao*. *Theobroma cacao* which is cultivated in the tropical regions of the world originates from the Amazon region of South America. Cocoa beans processing involves two main steps, which are fermentation and drying. In Ghana, cocoa is the major export crop. The country enjoys a price premium on the international market as it is widely known for its quality cocoa beans. The cocoa industry, creates employment, generates incomes and earns foreign exchange for the nation. This makes it very important for producing countries to produce more for export. Due to the uses of cocoa, consuming countries, which do not have suitable climates for cocoa production, import more. This has resulted in a global cocoa beans supply chain, with a production process that involves farmers, buyers, shipping organizations, processors, manufacturers and distributors (ICCO, 2013).

The search for alternative sources of oil from non-commercial plant sources such as cocoa has become imperative. The oil of cocoa (*Theobroma cacoa*) is noncommercialized and was therefore selected for this study. Studies have been conducted on the oil composition of cocoa beans and its usage in food products. Through these studies, it has been established that cocoa butter and margarine can also be produced from cocoa beans with its quality being equivalent to that produced from other vegetable oils. Cocoa beans in its original state have limited commercial uses (ICCO, 2013).

Smallholder cocoa farmers over the past years have been selling their produce in the form of raw cocoa beans. This makes the farmers depend solely on the marketing of the raw cocoa beans for income. This is a major challenge facing farmers since they do not add

value to the raw cocoa beans to get additional income by processing the cocoa beans into diverse forms to serve as an alternative source of income from their produce. Farmers therefore have no option than to rely on the buyers of their cocoa beans.

Worldwide, Africa is seen to have the capacity to produce raw materials for edible oil. In order for Ghana to benefit fully from this rare opportunity, raw materials should be processed into edible oil for export with a corresponding increase in income. In addition, employment will be created in the country and continent at large. All these cannot be done without serious research on edible oil production. Oil has been produced from cocoa under varied conditions from a handful of sources; hence the best method and conditions for production should be determined (Chhetri *et al.,* 2008). The use of oil quality parameters will help establish the best fermentation duration and drying method from cocoa compared to other sources. This study thus sought to produce cocoa bean oil and analyze its quality in accordance with existing oil standards. This work therefore produced cocoa bean oil and analyzed its quality using established oil quality standards and also determined the effect of different fermentation duration and different drying methods on the quality of cocoa bean oil.

The specific objectives were to:

1. assess the effect of the different fermentation duration on the chemical and physical properties of the oil produced from cocoa;
2. assess the result of different methods of drying on the physical and chemical quality of the oil produced from cocoa; and
3. determine which fermentation duration and drying methods produce the most suitable oil.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ORIGIN OF COCOA

Cocoa (*Theobroma cacao* L.) belongs to the family *Sterculiaceue.* In the 18th century the Swedish botanist, *Carolus Linnaeus*, renamed the cocoa tree giving it the Greek name *Theobroma Cacao*, now its official botanical name, which literally means 'food of the Gods'. They grow best under the canopy of tropical rainforests, seldom reaching more than 7.5 metres (25 feet) high. Cocoa is a major agricultural commodity grown exclusively in tropical areas in three principal producing regions namely Africa, Latin America and the Caribbean, Asia and Oceania. At the start of the 20th century, Latin America and the Caribbean region accounted for 60% to 80% of the world's supply of cocoa beans (Sukha, 2003). History attributes the commercial cultivation of cocoa in Ghana to Tetteh Quarshie; a native who had travelled to Fernando Po and returned with

Amelonado cocoa pods (David and Darko, 2009). The Europeans also introduced it into West Africa and the rest of Asia. Commercial cocoa which is obtained from the beans originated as seeds from the ripe pods of the plant *Theobroma cacao*, which is native to the Amazon region of South America and cultivated in the tropical regions of the world (Ardhana and Fleet, 2003). Cocoa trees begin to bear fruit when they are three to four years old. They produce pink and white flowers throughout the year, growing in abundance after the rain starts. However, the pods grow straight out of the trunk and the main branches, which is most unusual. Only a small proportion of the flowers develop into fruit over a period of about five months. The trees are carefully pruned so that pods can be more easily harvested. Each tree yields 20-30 pods per year.

2.2 VARIETIES OF COCOA

According to Clapperton (1993), the type of planting material has a major influence on the flavour and colour of the cocoa beans. Broadly, cocoa can be classified under three major types, namely, Criollo, Forastero and Trinitario, which is hybrid of the first two.

Forastero- This is the most hardy and vigorous type of cocoa. In addition, its beans have the strongest flavor. It forms the largest part of all the types of cocoa grown (Tomlins *et al.,* 1993).

Amelonado is the variety of forastero that is grown mostly in Brasil and West Africa. It has a unique lightly furrowed (comparatively smooth) pod with beans which are pale purple. Forastero, is further grouped into Amazonia and Amelonado, with the former being grouped further into Upper Amazonia and Lower Amazonia based on its area of origin (Tomlins *et al.,* 1993).

Criollo–Trees are comparatively less hardy, producing pods which are red in colour and softer. Averagely, pods produced by Criollo trees contain 20-30 seeds/beans per pod. These beans are white, ivory or very pale purple. It has a flavour mild or weak chocolate. Criollo is predominantly grown in Central and South America and Indonesia. Sanchez, one of the varieties of Criollo is grown in the Republic of Domincan. Beans produced from this variety are fermented shortly or not fermented at all (Saltini *et al.,* 2013).

Trinitario-These plants are hybrids of Forastero and Criollo. These plants were obtained through breeding of the first two types of cocoa. This implies they do not originate from the wild. They are mostly cultivated in Cameroon, Papua New Guinea and the Caribbean. The pods contain an average of 30 or more seeds or beans. The beans are rarely white but vary in colour (Mossu, 1992).

2.3 USES OF COCOA

Cocoa is used in producing a variety of products. Broadly, cocoa is used in manufacturing beverages, cosmetics, pharmaceuticals, manure, and many more. Cocoa pod husk is used in the production of animal feed. The nutritional value of cocoa pod husk is very high, making it an alternative ingredient which can be used in the production of animal feed with a level of restriction (Adomako *et al.,*1999). Its usage is restricted due to one of the chemical components, theobromine being toxic at certain levels to livestock. Cocoa pod husk has been used as an ingredient in producing dry animal feed pellets which has successfully been substitute wheat bran to feed pigs and rabbits. Cameroon has also developed fish food partly from Cocoa pod husk which has proved to be a suitable alternative for feeding Tilapia (*Oreochromis niloticus*). All these alternative feeds produced with cocoa pod husk as an ingredient are cheaper, compared to the conventional feeds. Cocoa pod husk can also use in producing potash. This potash is used in manufacturing of soft soap, potash fertilizer and potash compost
(Opoku-Ameyaw *et al.,* 2010).

In developing countries such as Ghana, sources of protein and food and feed which are rich in energy are scarce. The few ones available are also fed on by man. Competition between man and animals for these same food sources has made them very expensive to be used for animal feed. This has made it imperative for man and specifically manufacturers of animal feed to fall on agricultural by-products which abound in large quantities for the manufacture of animal feed. In this regard, cocoa by-products stand tall as only the seeds or beans are used by most cocoa farmers. Hence the cocoa pod husk is used in producing both wet and dry animal feeds. The Cocoa pod husk is usually sliced before using it in feed production. Wet feed is used in the direct or on-farm feeding on day-

to-day basis in the course of the harvesting season whereas the dry feed is produced and stored for use, especially in the off-season (Opoku-Ameyaw *et al.,* 2010).

Cocoa pulp juice –This is also called "sweatings". This is the excess juice which drains off during fermentation of fresh cocoa beans. This may be collected as by-products with no adverse effect on the fermentation process. The juice is sterilized and bottled as soft drinks. In addition, the fresh pulp juice is fermented for 4 days and the alcohol distilled using a traditional distillation unit. The alcohol is afterwards refined and blended into gin and brandy. Again, the pulp juice is used in producing Jams and Marmalades (OpokuAmeyaw *et al.,* 2010).

Extraction of cocoa butter from discarded beans- Cocoa beans discarded are beans from pods which are rejected due to insect or rodents damage and blackpod infection. They also include mouldy, slaty or unfermented, germinated and flattened or small beans. Fat is extracted from them using the local extraction method of roasting, milling and boiling to extract the fat (Opoku-Ameyaw *et al.,* 2010).

Cocoa powder – It is used mainly as flavour in ice creams, cakes, dairy drinks and biscuits. Cocoa powder is also used by the beverage industry for the preparation of beverages such as milo (Opoku-Ameyaw *et al.,* 2010).

2.4 PROCESSING OF COCOA BEANS

Processing of cocoa beans involves a number of steps. These include:

2.4.1 Harvesting and Pod Plucking

Pod plucking is the first process undertaken by farmers after being convinced by the conventions that the cocoa is ready to be harvested. This stage involves the physical harvesting of the cocoa pods from the tree using the hook, knife or cutlass. The precaution here is that much care is taken to make sure that no physical damage is caused to the cocoa tree. Intensive labour is required in harvesting cocoa pods. In cocoa growing communities in West Africa, families and friends join hands and their combined labour is used to harvest their pods on rotational basis. In cases where they belong to farmer groups, some help themselves on rotational basis. Harvesting is done every 2-4 weeks in the main crop season when the pods are ripe, mostly yellow in colour. This is done by cutting the stalk of the fruit with a knife as close as possible to the pod in order not to cause injury to the tree. Cutlass is used to harvest pods within the reach the hands whereas pods out of the hands are harvested with sickle shaped knives on long poles. Again, extra care should be taken during harvesting to prevent damaging the cushion carrying the flowers and fruits (Mossu, 1992). Diseased pods and those damaged by insects and rodents are removed in the course of harvesting (Sukha, 2013).

The healthy pods are afterwards gathered at a central breaking point. The pods are then broken using either a wooding club or breaking knife. Wooden clubs are recommended and preferred as cutlasses may injure the beans inside the pod. Beans in the broken pod are scooped out whereas the placenta and husk are thrown away (Mossu, 1992). Beans which have germinated, black or diseased, pieces of husks and fragments of placenta are removed from the scooped beans (Mossu, 1992).

2.4.2 Fermentation

The sweet, white mucilageous pulp which the scooped beans are embedded in, serves as the substrate for fermentation. Fermentation starts from the day the pods are broken. Fermentation is done to remove or reduce the astringent and unpleasant flavour of the raw cocoa and to give it a good taste and pleasant flavor (Thompson *et al.,* 2001). The advantage of fermenting cocoa is to develop chocolate precursors in the bean. The pulp surrounding the bean undergoes fermentation but not the cocoa bean. Fermentation starts right after contamination of the pulp in the course of pod breaking with microorganisms from immediate environment such as pod knives, surfaces of pods and hands of workers. It is done in six days and the microorganisms involved are yeasts, lactic acid bacteria and acetobacter. The four main fermentation methods are heap, tray, box and basket methods. The simplest of the fermentation methods is the heap and it is therefore widely used in West Africa, especially in small holder farms. In heap fermentation, fresh plantain, banana or pawpaw leaves are spread on the ground in a circle. Drainage holes are created in the mat of leaves, through puncturing with a pointed stick to allow easy drainage of the pulp. The scooped beans are heaped on the mat and covered with more leaves. The heap is held in place by small logs. Turning of the beans is done after every 48 hours or 2 days. The beans are removed on the sixth day and drying begins (Thompson *et al.,* 2001).

Fermentation is affected by ripeness of pods, pod storage before breaking, quantity of beans and pulp during fermentation, turning of the fermenting mass, diseases that affect pods and seasonal or climatic effects (Jespersen *et al.,* 2005).

2.4.3 Drying

Drying starts the same day that fermentation ends. This is simply, reducing the moisture of the beans from about 55% to 7%. The beans after fermentation are carried to the drying area and spread thinly on raised mats. Stirring of the beans must be done frequently to pick out germinated, flat and black beans, any foreign material or placenta. Even drying is achieved through the use of the mats. Asphalt roads or bare floor usage in drying is not recommended. The above traditional method of drying is called sun drying. The other method of drying, mechanical drying is very expensive, there is also the risk of contaminating the beans with smoke and retaining high levels of acid in the beans, it is therefore not recommended. Drying of beans takes a period of about 10 -14 days depending on the atmospheric conditions and volume of beans per square area on drying mat. Well dried beans will crack easily when squeezed between the fingers and can easily be cut which could result in product deterioration. The essence of drying is to prevent product deterioration which may result from over fermentation (Amoah-Awuah *et al.,* 2006).

2.4.4 Bagging and Storage

The cocoa beans are packed in clean jute sacks after drying and cleaning them from extraneous materials. They are stored in a well-ventilated and a relatively low humidity environment to prevent re-humidification of the beans. They are afterwards transported to licensed buying companies for sale (Opoku-Ameyaw *et al.,* 2010).

2.4.5 Winnowing

When the dried beans get to the processing factories, air currents are used to separate the shells from the nibs after cracking the beans. The nibs are used in the manufacture of chocolate (ICCO, 2013).

2.4.6 Roasting

Roasting of the nibs is done in specialized ovens at 105-120 degrees of temperature. The duration for roasting the beans depend on what the cocoa is going to be used for. Roasting essentially darkens the cocoa nibs, giving it a brownie, rich colour and completes the chocolate aroma and flavour (ICCO, 2013).

2.4.7 Grinding

Grinding of the roasted nibs is done in stone mills. The nibs are continuously grounded until it becomes a thick chocolate-coloured liquid. The cocoa butter content of this liquid called “mass” ranges from a percentage of 53 to 58, making it solidify upon cooling. Manufacture of all cocoa products and chocolate start with this (ICCO, 2013).

2.4.8 Pressing

Pressing is done to extract cocoa butter from the cocoa mass using strong pressing machines. The cocoa left after extraction is processed into fine powder. This high quality powder is used for the production of beverages (ICCO, 2013).

2.5 DRYING OF COCOA BEANS

Cocoa beans drying involve heating of the beans with the aim of reducing the content of moisture in them to less than 7.5 % (W/W). Drying is complete when the farmer deems fit

that his beans are dry using his own indicators (Lainé, 2001). No matter how experienced and well trained the farmer is, the content of moisture may vary between the beans (Rohsius *et al.,* 2006). According to Bart-Plange and Baryeh (2003), the content of moisture varied in dried cocoa beans from 5% to 24% for 2000/2001 harvesting season. Oxidation in fermentation is continued by drying. Drying therefore helps in bringing down acidity, bitterness and astringency. Comparative studies on natural and artificial drying methods have proven that the best drying result is achieved through sun drying naturally (Bonaparte *et al.,* 1998).

Drying of cocoa beans can be achieved by natural or artificial means. The natural method of drying cocoa beans is done traditionally through sun drying. A very essential step in cocoa processing is drying, due to completion of chemical reactions which gives good flavour to the beans in the course of drying. It gives way for evapouration of acids and leads to lower content of acids in the cocoa, resulting in good flavour of cocoa. Fermenting the cocoa for more than 7 days can lead to mould contamination and would lower the value of the cocoa (Takrama and Adomako, 1996).

The bean water content, following fermentation, is about 60 %, and it must be reduced to less than 7% during drying to avoid spontaneous mould and bacterial growth under storage and transport (Takrama and Adomako, 1996). The essence of drying is to forestall over fermentation Drying of beans takes a period of about 10 -14 days depending on the atmospheric conditions and volume of beans per square area on drying mat. Well dried beans will crack easily when squeezed between the fingers and can easily be cut with a knife (Amoa-Awua *et al.,* 2006). The essence of drying is to prevent spoilage of the product which may be caused by fermenting beyond 7 days. Drying with sun is the natural means of drying beans in the sun on raffia mats. It is simple and cheap but it is also labor-intensive and there is much concern for a stable weather condition. In sun drying, cocoa beans are

shielded from materials other than cocoa and animals through their platforms which are elevated and the mats put on them. The mats are placed on a raised platform to protect the cocoa beans against animals and foreign materials. There is also the frequent stirring of beans on mat to facilitate drying. At night and whenever the rain comes, the mats should be rolled up (Are and Gwynne-Jones,

1974). Artificial dryers include the use of ovens; solar dries etc in drying cocoa beans. The beans are spread in trays, allowing the air to permeate through a ladder system (Mossu, 1992). Using this method, it is very important that the cocoa beans are not contaminated with smoke from the fire, since dry beans easily absorb flavours and aromas from the environment (Barclays Bank, 1970). Hii *et al.,* (2008), determined the kinetics of drying cocoa beans with natural and artificial drying methods and found that the temperature and relative humidity during the drying period fluctuated between 26ºC33ºC and 56%-82% respectively. Starting with beans with initial moisture content of 51% weight of beans (wb), they observed that the content of moisture reduced unwaveringly as duration for drying increased. Drying to 7% moisture content (mc), took 52 hours to end, using the artificial means whereas it took 73.5 hours to end using the natural means.

2.5.1 Some benefits of drying cocoa beans

Drying removes both water and excess acidity from the beans; it also ensures longer storage of the cocoa beans. The commonest method of drying in Ghana is the use of the sun drying. During drying, turning is done from time to time to ensure a uniform mixing and exposure of the beans to sunlight. There is a possibility of aerial deposition of some heavy metals onto the shells of the beans during drying (Newman, 2000).

2.6 FERMENTATION OF COCOA BEANS

Fermentation, another major stage of processing, helps in the removal of the pulp and induces the death of the bean before drying. In the course of fermentation, precursor of flavour formation starts, bitterness is reduced significantly and development of colour starts (Biehl *et al.,* 1990). According to Voigt *et al.,* (1994), disruptions in the cells and death of the seeds start enzymatic and non-enzymatic reactions among the components of seed. The enzymatic and non-enzymatic reactions impact on the beans colour and also produce a crop of precursors of flavour (Schwan *et al.,* 1995; Adeyeye *et al.,* 2010). In the course of roasting, these precursors experience additional transmutation which creates the ultimate compounds of chocolate flavour (Lopez and Dimick, 1991; Thompson *et al.,* 2007; Ouattara *et al.,* 2008; Rodriguez-Campos *et al.,* 2011).

For the formation of best flavour, organic acids rate of diffusion into the cotyledons, time of first entry, length of optimum pH period and last pH, are of extreme importance (Biehl *et al.,* 1985). Higher pH beans (5.5-5.8) are looked at as unfermented, with its accompanying low index of fermentation and score for cut test. On the contrary, beans considered as well-fermented are the ones with lower pH (4.75-5.19). Good techniques in fermentation can maximize the flavours of chocolate through the reduction of acid (Holm *et al.,* 1993; Beckett, 2008).

Well-fermented and dried quality beans have low bitterness and astringency, a good brown colour and off-flavours such as smoky are absent (Afoakwa and Paterson, 2010).

Cocoa beans fermentation happens in two phases: Microbial reactions occur in the pulp and the beans external part of the beans during the first phase, and in the final phase, hydrolytic reactions take place in the cotyledons (Biehl *et al.,* 1985; Schwan and Wheals, 2004). The activities of microbes in the pulp is an impermanent taking over by yeast in the

course of the early hours that is dominated by yeast, lactic acid bacteria (LAB) comes after, which reduces after fermentation for 48 hours, they are afterwards taken over by acetic acid bacteria(AAB). Bacillus species (e.g. *Bacillus subtilis*, *Bacillus megaterium*, and *Bacillus flexus*), filamentous fungi, and other bacteria may also grow throughout fermentation and bean flavour and quality can be affected (Ardhana and Fleet, 2003; Schwan *et al.,* 1995; Schwan and Wheals, 2004). Varieties of species of yeast have been made known to be cocoa fermentation colonizers. The genera that have been found predominantly are *Hanseniaspora (Anamorph kloeckera*), *Pichia* and *Saccharomyces.* According to Pereira *et al.,* (2012), *Saccharomyces cerevisiae* attained maximum population of 107 CFU/g at 24 h in the course of box fermentation of cocoa. Afterwards, a variety of other species with *Pichia kudriavzevii (anamorph, Candida krusei*, also previously described as *Issatchenkia orientalis)* as an example, overshadows *Saccharomyces cerevisiae*.

Farmers use different methods of fermentation to ferment their cocoa based on their locality and countries (Lainé, 2001). The notable methods of fermentation are box, tray, platform and heap. The most popular fermentation procedure in Ghana is the heap method. The heap method involves heaping beans which have freshly been harvested on plantain or banana leaves and covering the beans further with extra leaves. Normally, fermentation is completed in about 6 days. To ensure uniform fermentation of the beans, the heap is turned and mixed on the 2nd and 4th days within the 6 days of fermentation. After fermentation, drying is employed in order to make the beans ready for export or industrial use. Generally, box fermentation produces cocoa beans with sugar, acetic acid and ethanol in comparatively reduced concentrations and a comparatively high pH. The rate of increase in temperature when fermentation starts is relatively slower in box fermented cocoa beans. This method has been seen to produce reduced uniformity in several instances, which can

lead to increased number of beans which are defective or sugars not completely used (Guehi *et al.,* 2010; Howat *et al.,* 1957; Tomlins *et al.,* 1993). It has been established that Tannins, presence of purple beans, sugar content and pH is substantially affected by the material used in construction, shape and size of the box (Guehi *et al.,* 2010; Portillo *et al.,* 2007). There is a quicker rise in temperature during the initial stage of heap formation than box fermentation, which leads to a more uniform fermentation (Tomlins *et al.,* 1993). This may be the cause of reduced purple and more beans which are brown being recorded in the heap fermentation method than the box fermentation (Guehi *et al.,* 2010). On the contrary, some Scientist also recorded no difference between the two aforementioned methods (Carr *et al.,* 1979). Platform method is generally seen as a disused method of fermentation (Thompson *et al.,* 2001). On the contrary it is still used in most cocoa growing regions such as West Africa due to its cost being cheap (Lainé, 2001). Fermentation rate for beans fermented on platforms is generally low. May be this is why Criollo beans were fermented using this method in the olden days. This is because; Criollo needs short fermentation (about 2 or 3days). On the contrary, it cannot be used for drying Forastero beans, as the latter needs longer period of fermentation (5-8days). With Forastero cultivar, the use of the platform stimulates unwanted growth of moulds and the resultant off-flavours development (Thompson *et al.,* 2001).

2.7 NUTRITIONAL COMPOSITION

Dried cocoa beans has 2.58 - 4.26% moisture content, 13.19 – 14.42% crude protein, 33.93-39.21% fat, 2.52- 2.66% fibre, 3.19-4.10% ash and 38.12- 40.17% carbohydrate content (Amankwaah, 2013). Cholesterol in the blood is not increased by Chocolate and

Cocoa butter. On the other hand, lower grade chocolate consumption, where the fat content originates from milk fat or other types of fat, it adversely affect the cholesterol level. Cocoa beans are a rich source of, calcium, zinc, iron, potassium, manganese, copper and magnesium, which are essential minerals. It is also a rich source of vitamins such as panthotenic acid and vitamins A, B1, B2, B3, C and E (Amoah, 2013).

Table 2.1: Proximate composition of by-products from the manufacture of cocoa.

Proximate	Cocoa pod husk	Cocoa bean shell	Cocoa bean meal
Moisture	5.4-15.3	4.9-12.0	2.1-16.9
Crude protein	6.3-10.4	13.2-20.1	17.8-28.6
Crude fibre	23.4-36.2	9.3-20.5	5.3-22.0
Ether extractable components	0.5-2.4	1.9-22.0	1.1-17.8
Nitrogen-free extract	31.8-61.4	40.2-52.5	25.9-51.1
Ash	7.0-15.3	6.0-10.8	3.0-15.1

Source: The EFSA Journal (2008).

The differences in the proximate composition could be caused by varying the varieties being studied annually and variations in the samples prepared for analysis and the method of analysis used in analyzing the data (Alvarado *et al.,* 1983).

2.8 QUALITIES OF OIL

There are a lot of ways the evaluation of the quality of oil may be done. Assessment of quality using senses to evaluate foods such as taste, may be the most important, but they are not practical in the control of quality. Quantitative methods are preferred so that points

of rejection could easily be found through the use of our senses (Warner, 2002). One procedure in all cases cannot give authentic results, because of how complex the problem is. A lot of procedures have been formulated based on chemical and physical parameters to find out the quality of oils and fats used in deep frying. Analytical methods conventionally used includes titrimetric for free fatty acids (FFA), chromatographic for total polymer content, peroxide value, iodine value determinations, saponification value, phenolics and specific gravity (AOAC, 2000).

2.8.1 Free Fatty Acid (FFA)

A decline in the quality of oils is normally indicated by free fatty acids. This is the indicator used by most food processors because it is comparatively fast and the most reliable when the acidity of oils are monitored during frying (Tarmizi and Ismail, 2008). Free Fatty Acids represents the percentage of fatty acids liberated from the triglyceride chain during heating due to hydrolysis and oxidation (Nor *et al.,* 2008). Free fatty acid formation is linked with smoke point; FFA is inversely proportional to the smoke point (Tarmizi and Siew, 2008; Sulieman *et al.,* 2006). observed that in the course of frying, free fatty acid values reduced after the 4^{th} and 12^{th} hours. This may be attributed to the volatilization of fatty acids with lower molecular weight. In the course of transesterification, content of moisture and FFA are major parameters used in finding out vegetable oil viability. In order to finish the reaction catalyzed; an FFA value below 3% is required. The oil's acidity is inversely proportional to the conversion efficiency (Dorado *et al.,* 2002). According to Ma *et al.,* (1998), in the study of NaOH catalyzed beef tallow transesterification, beef tallow methyl esters (BTME) recorded the highest yield without the addition of water and FFA. BTME yielded the least value, below 5%, with the addition of 0.6% of free fatty acids and with the addition varying levels of water. Again, it yielded about 17% with the addition of 0.9%

of water, with no FFA addition. Therefore, to produce biodiesel fuel from the vegetable oil or beef tallow, they should be processed by using NaOH solution to saponify them. This will remove FFA which is very high in the vegetable oil and beef tallow. In addition, the free fatty acids can be esterified using the acid catalyzed process.

2.8.2 Acid Value

The oil's acid value indicates the free fatty acid quantity contained in it. Acid value should therefore not be very high, as it will indicate a high free fatty acid content. Free fatty acid content of oil is directly proportional to its acid value. For the vegetable oil, the acid value should be below 1 to enhance the catalyzed transesterification process (Ellis *et al.,* 2007).

According to Van Gerpen (2005), an alkali can be used to catalyse the reaction to increase the FFA content, but not above 5%. Again, it was also reported that, for oils with the content of FFA higher than 3% the process of transesterification would not occur. It has also been established that the yield of transesterification reduces when the percentage composition of FFA is greater than 5%. This suppresses methoxides formation through the partial neutralization of existing catalyst, which brings forth soap in the medium of reaction. The amount of free acids present in a sample is called Acid value. It is mostly used as an indicator in checking the quality of oils used in frying.

2.8.3 Saponification Value

The soap making ability of an oil is simply its saponification value. It is a measure of the average molecular weight of all triacylglycerols a sample of oil contains. Triglycerides are usually indicated by a high value of saponification. Oils with high saponification value are good for manufacturing shampoo and soaps which are liquid. Palm oil, Palm kernel oil and

Jatropha curcas oil with saponification values of, (201.0 mg/KOH/g), (247.0 mg/KOH/g) and (202.6 mg/KOH/g) respectively, are examples of oils which have high values of saponification. Hence, these oils might be good for the manufacturing of soap (Ankapong, 2010).

2.8.4 Iodine value

This is the measure of the level of unsaturation (the number of carbon to carbon double bonds) of oils and fats. In practical terms, the grams of iodine absorbed per a sample of 100g of oil are iodine value. Iodine value is directly proportional to the level of unsaturation. This implies the higher the iodine value of fats and oils, the higher the level of unsaturation in them (Food Analysis, 4th Edition). Iodine value reduces through heating due to oxidation. The rate of oxidation increases in the course of frying. This is seen in the differences in the iodine values during that period. According to Augustin and Berry (1983), when oil deteriorates excessively, the value of iodine is also seen to change significantly.

2.8.5 Refractive index

The ratio of the speed of light in air (vacuum) to the speed of light in an oil or fat is the refractive index of the oil or fat. Refractive index is directly proportional to the level of unsaturation. Hence, refractive index reduceses linearly as iodine value also reduces. Refractive index is used to assess the level of purity and a way of identification of an oil. This is because each fat or oil has a unique refractive index (Food Analysis, 4th Edition).

2.8.6 Phenolics

Phenols which are also called phenolics are chemical compounds which are detected in food. Typical examples of phenols are acetyladehyde, citric acid, quericitin and caffeine. Hormones and neuro-chemicals are other examples of phenolics (Pourmorad *et al.,* 2006).

2.8.7 Peroxide Value

Peroxide value is determined by redox titration. The primary proof of unsaturated fats and oils rancidity is peroxide value. Peroxide value gives a degree of the temporal product of oxidation, such as, after foaming. In spite of its challenges it is one of the commonest lipid oxidation tests. The degree to which a sample of oil has experienced primary oxidation is shown by peroxide value and p-anisidine test might also be used to ascertain secondary oxidation (Cheman and Wanhussin, 1998).

According to Cheman and Wanhussin (1998), peroxide value of vegetable oils with a high quality should be less than 2meq/kg. Autoxidation is influenced by double bonds present in oils and fats. The higher the unsaturation level of fats and oils, the more liable they are to autoxidation. Again, autoxidation is a chemical reaction which occurs freely using oxygen. This spoils fats and oils which produces off-odours and off-flavours.

Fats and Oils with peroxide values higher than 20 are poor in quality. This is normally shown in their off-flavours. Peroxide value is therefore very important in determining the level of deterioration of fats and oils (Cheman and Wanhussin, 1998).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL LOCATION

The fermentation and drying of cocoa beans was carried out at the Department of Horticulture, KNUST and the determination of yield and oil quality parameters was also carried out at the Laboratory of Department of Food Science and Technology, KNUST.

3.2 SAMPLE COLLECTION

Fully riped cocoa of mixed varieties was harvested from experimental fields of (CHED), Obuasi Cocoa District and brought to the Department of Horticulture for heap fermentation (5 and 7 days) using 10 kg of cocoa beans for each treatment. Sun and solar drying methods was used.

3.3 TREATMENT COMBINATIONS

- Treatment combinations were:
- Heap fermentation for 5 days + Sun drying.
- Heap fermentation for 5 days + Solar drying.
- Heap fermentation for 7 days + Sun drying.
- Heap fermentation for 7 days + Solar drying.

3.4 EXPERIMENTAL DESIGN

The experiment design adopted for this study was a 2 by 2 Factorial in Completely Randomized Design. Duration of fermentation and drying methods were the two factors tested at two levels, 5 days and 7 days, then sun and solar drying, respectively.

3.5 SAMPLE PREPARATIONS AND OIL EXTRACTION

Six hundred (600) grams (g) of dried cocoa seeds were milled using a hammer mill and passed through a 1.18 mm standard sieve to obtained fine particle size (Ellis, *et al.,* 2007).

A 2.0 g sample was transferred into a paper thimble, stuffed with non- absorbent cotton. The thimble containing the sample was then placed in the extractor of the Soxhlet apparatus. A clean dried Soxhlet flask was weighed and about 240 ml of petroleum ether was measured into it. The Soxhlet apparatus was then set up. Cold water was turned on so it could pass through the condenser and help condense the organic solvent. The set up was left to run 3 to 4 hours (h). The extraction flask was dismantled and allowed to cool. The excess ether was evaporated over water bath until no odour of ether remained. The extraction flask was allowed to cool and the weight of the extract determined (AOAC, 2002).

$$\% \text{ ether extract} = \frac{\text{ether extract weight}}{\text{Sample weight}} \times 100$$

3.6 LABORATORY ANALYSIS

3. 6. 1. Oil Qualities Determination (Cocoa flour)

The following oil quality parameters were assessed; peroxide value, total phenolic, saponification value, iodine value, acid value, refractive index, pH, relative density and free fatty acid were determined.

3. 6. 1 .1 Perioxide value

Thirty milliliters acetic acid-chloroform solution (3:2 v/v) was added to a 5grams (g) sample into a 250 (millilitres) ml glass stopper Erlenmeyer flask and heated gently. 0.5 ml of saturated potassium iodide solution was added and the content was swirled for one minute followed by the addition of 30 ml distilled water with vigorous shaking to liberate the iodine from the chloroform layer. The content was titrated against 0.1 N sodium

thiosulfate using, 1 ml of starch solution as indicator. The titration was done until the blue gray colour disappears in the aqueous (AOCS, 2006).

3. 6. 1 .2 Total phenolics

Five hundred (500) milligrams (mg) of gallic acid was weighed and dissolved with 90% ethanol solution. 20% w/v Na_2CO_3 was prepared by dissolving 20 g anhydrous sodium carbonate in 100 ml distilled water. The total phenol content of samples were analyzed using Folin-Ciocalteau method by Singleton and Rossi (1965), as used by Gardner *et al.,* (2000), and adapted by Bailey (2007), with slight modifications. The Folin-Ciocalteau method is based on measuring the colour change from yellow to blue as a result of reduction of the *tungstate-molebdate* mixture in the *Folin-Ciocalteau* reagent by phenols present in the analyte solution. 0.1 ml of sample or distilled water as blank was pipetted into 10 ml volumetric flask. 6 ml of distilled water added followed by 0.5 millilitres (ml) *Folin-Ciocalteau reagent*. 1.5 ml of 20% sodium carbonate solution was added, mixed and allow to stand for 5 minutes. This solution was topped with distilled water to the 10 ml mark and mixed thoroughly. After incubating the resulting solution for 2 hours at room temperature, its absorbance was measured at 750 nm using Thermo Scientific Nanodrop spectrophotometer ND-1000. For each sample, triplicate absorbance readings were taken. A serial dilution of 0, 50, 100, 150, 250 and 500 mg/l respectively was prepared. The procedure for analyzing each sample was repeated for each serial dilution. Phenol content was expressed as concentration of gallic acid equivalent (GAE, mg/l) using equation of line of best fit obtained from the standard calibration curve.

3. 6. 1 .3 Saponification value

Two (2) gram (g) sample was dissolved with 25 millilitres (ml) of alcoholic potassium hydroxide solution (0.5 N) inside a 250 ml conical flask. A reflux condenser was attached

and the flask heated with its contents on a boiling water bath for 1 hour with occasional shaking. Using phenolphthalein as indicator, the excess potassium hydroxide was titrated with the 0.5 N hydrochloric acid. The procedure was repeated for an empty conical flask as blank and the saponification value determine from the two procedures (Ogbunugafor *et al.,* 2011).

3. 6. 1 4 Iodine value

Two (2) gram (g) of sample was weighed and dissolved in a solution of 5 ml of chloroform and 5 ml of Dan's reagent. This was done in a fume hood and kept for 10 mins. 5 ml of 10% potassium iodite was added followed by 20 ml distilled H_2O. The mixture was stirred several times and then titrates to a colourless end point with 0.025N $Na_2S_2O_3$ (Eromosele *et al.,* 1994).

3. 6. 1 5 Acid value

One (1) gram (g) of sample was dissolved with a 50 ml mixture of ether and ethanol (95% v/v) in a conical flask. The free acids in the samples were neutralized by titration against 0.1 N potassium hydroxide solution using phenolphthalein as indicator. The acid value was expressed as KOH g^{-1} (Ogbunugafor *et al.,* 2011).

3.6.1.6 Colour

The spectrophotometric method was used for the oil colour determination. The individual samples were pre-heated gently in a water bath. Using the Fisher Scientific Nanodrop Spectrophotometer, the absorbance of each sample was measured in triplicate for each one of wavelengths 460, 550, 620 and 670 nm, respectively. The photometric colour index was calculated with the American Oil Chemists Society (AOCS) methods

(AOCS, 2006).

3. 6. 1 .7 Refractive index

Extracted fat samples were slightly heated in a water bath to completely melt to liquid. The refractive index of each sample was determined with a refractometer at 20 °C. After calibrating the refractometer (Reichert digital refractometer, 13950000, USA) with distilled water. About four drops of the oil samples were placed onto the cleaned, dry prism surface the button "READ" was pressed and the value recorded. (Ogbunugafor *et al.,* 2011).

3. 6. 1 8 pH

The pH of each sample was determining using the pH meter, (Mettler Toledo, 51302910, USA). The probe was put into a beaker containing liquid fat samples then "READ" was pressed on the meter and the results recorded (AOAC, 1990).

3. 6. 1. 9 Specific gravity

A pre-cleaned and dried 50 ml specific gravity bottle was weighed initially as W_1. The bottle was filled with distilled water and its new weight measured as W_2. The content in the bottle was emptied and pre-dried. The bottle was then filled with oil and weight determined as W_3 (Gunstone, 2004).

3. 6. 1.10 Free fatty acid

Five (5) g of sample was weighed into a 250 (millilitres) ml Erlenmeyer flask. The weighed sample was dissolved with 50 ml of neutralized ethanol by swirling with gently heating. Few drops of Phenolphthalein indicator solution or 0.5 ml 1.0 % (w/v)

phenolphthalein in 1-propanol were added. This was titrated against 0.1 N KOH with the end point colour change from colourless to pink. The mls of titrant was recorded (AOCS, 2006).

3.7 DATA ANALYSIS

All data collected was analysed with the Statistix 9 statistical Package. Mean were compared by running two-way ANOVA at 99% confidence interval using the LSD posthoc.

CHAPTER FOUR 4.0

RESULTS

4.1 OIL QUALITY INDICES OF COCOA BEANS

4.1.1 The effect of different drying methods on the acid value of cocoa bean oil. Solar drying of cocoa beans resulted in an acid value content of 2.95, whilst sun drying recorded (2.48), with no significant differences ($p>0.01$) observed.

Table 4.1: The effect of different drying methods on the acid value of cocoa bean oil

Drying methods	Mean values

Solar	2.95a*
Sun	2.48a
$LSD_{0.01}$	1.04
CV	19.95

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.1.2 The effect of different fermentation durations on the acid value of cocoa bean oil

The highest acid value content (3.44) was observed in the 7-days-fermentation treatment whiles the 5-day fermentation had the least (1.99). Significant differences ($p<0.01$) were observed among the two fermentation duration.

Table 4.2: Effect of different fermentation durations on the acid value of cocoa bean oil

Fermentation duration	**Mean values**
5 days of fermentation	1.99b*
7 days of fermentation	3.44a
$LSD_{0.01}$	1.04
CV	19.95

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.1.3 The interactive effect of the different drying methods and different fermentation durations on the acid value of cocoa bean oil.

Results on interaction between drying methods and fermentation duration on the acid value revealed that cocoa beans fermented for 7 days and solar dried had the highest acid value content, (4.01) and was followed by beans fermented for 7 days and sun dried with no significant difference ($p>0.01$) occurring. No significant differences ($p>0.01$) were observed between cocoa beans that were fermented for 5 days and were solar and sun dried. The least acid value content, (1.88) was observed in cocoa beans that were fermented for a period of 5 days and solar dried.

Table 4.3: Interactive effect of drying methods and fermentation duration on the acid value cocoa bean oil

Drying methods and Fermentation duration	**Mean values**
Solar x 5 days	1.88b*
Sun x 5 days	2.09b
Solar x 7 days	4.01a
Sun x 7 days	2.86ab
LSD $_{0.01}$	1.48
CV	19.95

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.1.4 The effect of different drying methods on the iodine value of cocoa bean oil

Sun dried cocoa beans recorded the highest (30.53) iodine value whiles solar dried cocoa beans had (26.46) with significant differences ($P<0.01$) occurring between them.

Table 4.4: Effect of different drying methods on the iodine value of cocoa bean oil

Drying methods	**Mean values**
Solar	26.46b*
Sun	30.53a
LSD $_{0.01}$	2.37
CV	4.29

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.1.5 The effect of different fermentation durations on the iodine value of cocoa bean oil

Cocoa beans fermented for 7-days recorded the highest iodine value (33.17) whiles the least iodine value (23.82) was observed in cocoa beans fermented for a 5-day period. Significant differences (P<0.01) were observed between them.

Table 4.5: Effect of different fermentation durations on the iodine value of cocoa bean oil

Fermentation duration	Mean values
5 days of fermentation	23.82b*
7 days of fermentation	33.17a
$LSD_{0.01}$	2.37
CV	4.29

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.1.6 The interactive effect of different of drying methods and different fermentation durations on the iodine value of cocoa bean oil

The highest iodine value (36) was observed in cocoa beans fermented for 7 days and sun dried and was followed by 7 day fermentation with solar drying of beans with significance difference (P<0.01) occurring. The lowest iodine value (22.59) was observed in cocoa beans fermented for 5 days with solar drying. No significant difference (p>0.01) were observed between cocoa beans fermented for 5 days and dried using both sun and solar drying methods.

Table 4.6: The interactive effect of different drying methods and different fermentation durations on the iodine value of cocoa bean oil

Drying methods and Fermentation duration	Mean values
Solar x 5 days	22.59c*
Sun x 5 days	25.04c
Solar x 7 days	30.33b
Sun x 7 days	36.00 a
$LSD_{0.01}$	3.35
CV	4.29

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.1.7 Different Drying Methods on the pH value of cocoa bean oil

Solar drying of cocoa beans recorded the highest pH value (4.43) sun drying method had the least pH value (4.39). Significant differences ($P<0.01$) were observed among the different drying methods.

Table 4.7: Effect of drying methods on the pH value of cocoa bean oil

Drying methods	Mean values
Solar	4.43a*
Sun	4.39b
$LSD_{0.01}$	0.02
CV	0.23

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.2. Fermentation Duration on the pH value of cocoa bean oil

Fermentation of cocoa beans for a period of 5 days resulted in the highest pH value

(4.43) of the cocoa oil whiles the least was found in cocoa beans that ware fermented for 7 days. Significant difference (P<0.01) was observed among the different fermentation durations.

Table 4.8: Effect of fermentation duration on the pH value of cocoa bean oil

Fermentation duration	**Mean values**
5 days of fermentation	4.43a*
7 days of fermentation	4.39b
LSD $_{0.01}$	0.02
CV	0.23

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.2.1 Effect of drying methods and fermentation duration on the pH value of cocoa bean oil

The pH values of the cocoa oil were in the range of 4.41 - 4.46 as shown in Table 4.19

The highest pH value was observed in cocoa beans fermented for 7 days with solar drying whiles the least pH value (4.33) was from cocoa beans fermented for 7 days and sun dried with Significant difference (P<0.01) occurring.

Table 4.9: Interactive effect of drying methods and fermentation duration on the pH value of cocoa bean oil

Drying methods and Fermentation duration	**Mean values**
Solar x 5 days	4.41b*
Sun x 5 days	4.41b
Solar x 7 days	4.46a
Sun x 7 days	4.33 c
LSD $_{0.01}$	0.03

CV	0.23

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.2.2 Effect of different drying methods on the refractive index of cocoa bean oil

Solar drying method recorded a refractive index value of 68.53 for cocoa oil whiles that of sun drying had a value of 68.43, with no significant difference ($p>0.01$) observed (Table 4.10).

Table 4.10: Effect of drying methods on the refractive index of cocoa bean oil

Drying methods	**Mean values**
Solar	68.53a*
Sun	68.43a
$LSD_{0.01}$	0.21
CV	0.16

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.2.3 Effect of different fermentation duration on the refractive index of cocoa bean oil

Fermentation of cocoa beans for a period of 7 days recorded the highest refractive index value of 68.56 for cocoa oil whiles fermentation for 5 days also had a value of 68.40. There was no significant difference ($p>0.01$) between the different fermentation durations (Table 4.11).

Table 4.11: Effect of fermentation duration on the refractive index of cocoa bean oil

Fermentation duration	**Mean values**
5 days of fermentation	68.40a*
7 days of fermentation	68.56a

LSD $_{0.01}$	0.21
CV	0.16

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.2.4 Effect of the interactive effect of different drying methods and different fermentation durations on the refractive index of cocoa bean oil

The refractive indices of the oil were observed to be in the range of 68.30-68.56. There were no significant differences ($p>0.01$) between the various factors (Table 4.12).

Table 4.12: The interactive effect of different drying methods and different fermentation durations on the refractive index value of cocoa bean oil

Drying methods and Fermentation duration	**Mean values**
Solar x 5 days	68.50a*
Sun x 5 days	68.30a
Solar x 7 days	68.56a
Sun x 7 days	68.56a
LSD $_{0.01}$	0.29
CV	0.16

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.2.5 Different Drying Methods on the saponification value of cocoa bean oil Cocoa bean oil extracted from sundried cocoa beans had a saponification value of 2.79 whereas oil from solar dried beans had 2.76, with no significant difference between them ($p>0.01$).

Table 4.13: Effect of drying methods on the saponification value of cocoa bean oil

Drying methods	**Mean values**
Solar	2.76a*
Sun	2.79a
$LSD_{0.01}$	0.84
CV	15.71

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.2.6 Fermentation Duration on the saponification value of cocoa bean oil Cocoa bean oil from fermentation of cocoa beans for a period of 5 days had a saponification value (2.97), whiles oil from 7-days fermentation of cocoa beans had 2.57, with no significant difference ($p>0.01$) occurring.

Table 4.14: Effect of fermentation duration on the saponification value of cocoa bean oil

Fermentation duration	**Mean values**
5 days of fermentation	2.97a*
7 days of fermentation	2.57a
$LSD_{0.01}$	0.84
CV	15.71

**Mean values are not significantly different when they are assigned the same letter within a column.*

4.1.2.7 Effect of drying methods and fermentation duration on the saponification value of cocoa bean oil

The saponification values of the cocoa bean oil were in the range of 2.23 - 3.35 (Table 4.15) There were no significant difference ($p>0.01$) between the various factors.

Table 4.15: The interactive effect of the different drying methods and different fermentation durations on the saponification value of cocoa bean oil

Drying methods and Fermentation duration	**Mean values**
Solar x 5 days	2.60a*
Sun x 5 days	3.35a
Solar x 7 days	2.92a
Sun x 7 days	2.23a
$LSD_{0.01}$	1.19
CV	15.71

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.2.8 Different Drying Methods on the free fatty acid content of cocoa bean oil

Solar drying method recorded the highest free fatty acid content whiles that of sun drying had (1.24) with no significant difference ($p>0.01$) occurrence.

Table 4.16 Effect of drying methods on the free fatty acid content of cocoa bean oil

Drying methods	Mean values
Solar	1.39a*
Sun	1.24a
$LSD_{0.01}$	0.54
CV	21.28

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.2.9 Effect of different fermentation durations on the free fatty acid content of cocoa bean oil

The highest free fatty acid content of 1.63 was recorded in the oil from cocoa fermentation duration of 7 days whiles oil from fermentation for 5 days had free fatty acid content of 1.02. There was significant difference ($p<0.01$) between the two treatments.

Table 4.17: Effect of fermentation duration on the free fatty acid content of cocoa bean oil

Fermentation duration	Mean values
5 days of fermentation	1.02b*
7 days of fermentation	1.63a
$LSD_{0.01}$	0.54
CV	21.28

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.3 The interactive effect of different drying methods and different fermentation durations on the free fatty acid content of cocoa bean oil

The free fatty acid content of the cocoa bean oil was in the range of 0.92 - 1.88. Cocoa beans fermented for 7 days and solar dried had the highest free fatty acid content of 1.88 and was followed by fermentation for 7 days with sun drying whiles the least (0.92) was reported on fermentation for 5 days with solar drying.

Table 4.18: Interactive effect of drying methods and fermentation duration on the free fatty acid content of cocoa bean oil

Drying methods and Fermentation duration	**Mean values**
Solar x 5 days	0.92b*
Sun x 5 days	1.12ab
Solar x 7 days	1.88a
Sun x 7 days	1.37ab
$LSD_{0.01}$	0.77
CV	21.28

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.3.1 Effect of different drying methods on the relative density of cocoa bean oil

Solar drying method recorded a relative density of 0.88 whereas sun drying also recorded

a relative density of 0.86. There was no significant difference (p>0.01) between the treatments means of the oil produced from the different drying methods.

Table 4.19:The effect of different drying methods on the relative density of cocoa bean oil

Drying methods	**Mean values**
Solar	0.88a*
Sun	0.86a
LSD $_{0.01}$	0.02
CV	1.15

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.3.2 Effect of different fermentation Durations on the relative density of cocoa bean oil

There were no significant difference (p>0.01) occurrence between the treatments as the two fermentation durations all recorded (0.87) as shown in table 4.20.

Table 4.20: Effect of fermentation duration on the relative density content of cocoa bean oil

Fermentation duration	**Mean values**
5 days of fermentation	0.87a*
7 days of fermentation	0.87a
LSD $_{0.01}$	0.02
CV	1.15

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.3.3 The interactive effect of different drying methods and different fermentation durations on the relative density of cocoa bean oil

The relative density of cocoa oil ranged from 0.86 to 0.88 (Table 4.21). There were no significant difference ($p>0.01$) between the treatment means.

Table 4.21: Interactive effect of drying methods and different fermentation durations on the relative density of cocoa bean oil

Drying methods and Fermentation duration	Mean values
Solar x 5 days	0.88a*
Sun x 5 days	0.86a
Solar x 7 days	0.88a
Sun x 7 days	0.87a
$LSD_{0.01}$	0.02
CV	1.15

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.3.4 Effect of different drying methods on the peroxide value of cocoa bean oil

There were no significant difference ($p>0.01$) occurrence between the treatments as the two different drying methods all recorded a value of 21.33 (Table 4.22).

Table 4.22: Effect of different drying methods on the peroxide value of cocoa bean oil

Drying methods	Mean values
Solar	21.333a*
Sun	21.333a
$LSD_{0.01}$	4.4738
CV	10.83

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.3.5 Effect of different fermentation duration on the peroxide value of cocoa bean

oil

The peroxide value for the oil produced from cocoa beans fermented for 5-days was 23.33 whereas that of the 7-days had 19.33 with no significant difference ($p>0.01$) between them (Table 4.23).

Table 4.23:Effect of different fermentation duration on the peroxide value of cocoa bean oil

Fermentation duration	**Mean values**
5 days of fermentation	23.333a*
7 days of fermentation	19.333a
$LSD_{0.01}$	4.4738
CV	10.83

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.3.6 The effect of drying methods and different fermentation durations on the peroxide value of cocoa bean oil.

The highest peroxide value of 25.33 was observed in the 5 days fermentation with solar drying and the least of 17.33 was recorded on 7 days of fermentation with solar drying. There were no significant difference ($p>0.01$) occurrence between the treatment samples (Table 4.24).

Table 4.24: Interactive effect of different drying methods and different fermentation durations on the peroxide value of cocoa bean oil

Drying methods and Fermentation duration	**Mean values**
Solar x 5 days	25.333a*
Sun x 5 days	21.333ab
Solar x 7 days	17.333b

Sun x 7 days	21.333 ab
LSD $_{0.01}$	6.3270
CV	10.83

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.3.7 Effect of different drying methods on the total phenolic content of cocoa bean oil

The highest phenolic content of 1918.2 was recorded in the solar drying whiles sun drying had the least value of 68.8. There was significant difference ($p<0.01$) between the two treatments (Table 4.25).

Table 4.25: Effect of drying methods on the total phenolic content of cocoa bean oil

Drying methods	**Mean values**
Solar	1918.2a*
Sun	68.6b
LSD $_{0.01}$	76.018
CV	3.95

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.3.8 Effect of different fermentation durations on the total phenolic content of cocoa bean oil

The total phenolic content of the oil produced from cocoa beans fermented for 5-days was 1379.2 whereas that of the 7-days was 607.6, with no significant difference ($p>0.01$) between them (Table 4.26).

Table 4.26: Effect of different fermentation duration on the total phenolic content of cocoa bean oil

Fermentation duration	**Mean values**
5 days of fermentation	1379.2 a*
7 days of fermentation	607.6 a
$LSD_{0.01}$	76.018
CV	3.95

**Mean values are not significantly different when they are assigned the same letter within a column*

4.1.3.9 The interactive effect of the different drying methods and the different fermentation durations on the total phenolic content of cocoa bean oil

The total phenolic content of the cocoa bean oil ranged from 69.1 to 2690.3. Cocoa beans fermented for 5 days and solar dried had the highest total phenolic content of 2690.3 and was followed by fermentation for 7 days with solar drying whiles the least total phenolic content of 68.1 was recorded on 5 days fermentation with sun drying (Table 4.27).

Table 4.27: Interactive effect of drying methods and fermentation duration on the total phenolic content of cocoa bean oil

Drying methods and Fermentation duration	**Mean values**
Solar x 5 days	2690.3a*
Sun x 5 days	68.1c
Solar x 7 days	1146.0b
Sun x 7 days	69.1c
$LSD_{0.01}$	107.51
CV	3.95

**Mean values are not significantly different when they are assigned the same letter within a column*

4.2 YIELD OF OIL

4.2.1 Effect of different drying methods on the yield of cocoa bean oil

There were no significant differences between the treatment samples. The highest oil yield (29.00) was observed in the sun drying method with the least (28.20) obtained from the solar dried beans.

Table 4.28: Effect of drying methods on the yield of cocoa bean oil

Drying methods	**Mean values**
Solar	28.20[a*]
Sun	29.00[a]
Lsd (0.01)	3.484
CV	11.83

**Mean values are not significantly different when they are assigned the same letter within a column*

4.2.2 Fermentation duration on the yield of cocoa bean oil

A higher (28.00) yield of oil was observed in seven days fermentation whiles the lowest (24.10) was recorded on five days fermentation (Table 4.29).

Table 4.29: Effect of fermentation duration on the yield of cocoa bean oil

Fermentation duration	**Mean values**
5 days of fermentation	24.10b*
7 days of fermentation	28.00a

** Mean values are not significantly different when they are assigned the same letter within a column*

4.2.3 Effect of drying methods and fermentation duration on the yield of cocoa bean

oil

There was no significant difference between five days fermentation with both sun and solar drying and seven days fermentation with both sun and solar drying. The highest (28.20) oil yield was observed in fermentation duration for seven days with sun drying and was followed by seven days fermentation with solar dryer (27.100). The least (25.20) yield was also observed in five days fermentation with solar drying (Table 4.30).

Table 4.30: Interactive effect of drying methods and fermentation duration on the yield of cocoa bean oil

Drying methods and Fermentation duration	**Mean values**
Solar x 5 days	25.20b*
Sun x 5 days	26.00b
Solar x 7 days	27.10a
Sun x 7 days	28.20a

**Mean values are not significantly different when they are assigned the same letter within a column*

CHAPTER FIVE

5.0 DISCUSSION

5.1 ACID VALUE OF COCOA BEAN OIL

This study recorded an acid value of 2.95 for oil produced from cocoa beans that were solar dried and an acid value of 2.48 was also recorded for oil produced from cocoa beans that were sun dried with no significant difference between them. This shows that the method of drying had no effect on triglycerides hydrolysis and hence no effect on the acid value of the cocoa bean oil (Ellis *et al.,* 2007).

The lowest acid value (1.99) for fermentation duration was recorded for oil produced from the 5-day period whereas the highest acid value (3.44) was also recorded for the 7day fermentation period in table 4.2. This shows that the acid value content increases with an increase in fermentation duration.

As regards interaction between different methods of drying and different fermentation durations, 5-day solar drying recorded the lowest acid value (1.88) whilst 7-day solar drying recorded the highest acid value (4.01). This might be due to the duration or period of fermentation having an effect on hydrolysis of the triglycerides.

According to Codex Alimentarius (1984), the acid value of most edible oils ranges from 0-10. An oil extracted from *Treculia africana* seed recorded an acid value of 7.29 which falls within the edible oil range (Ellis *et al.,* 2007). In this study, the acid values obtained (1.88-4.01) was found to be within the acceptable limit (0-10) and therefore could be used in foods without apprehension.

5.2 IODINE VALUE (IV) OF COCOA BEAN OIL

Sun dried cocoa beans recorded the highest iodine value (30.53) whereas solar dried cocoa beans had the least IV (26.46) with significant differences ($P<0.01$) occurring between

them. The high iodine value could be due to a decline in oxidation resulting in high number of unsaturated bonds.

The lowest iodine value (23.82) for the fermentation duration was observed in the 5-days fermentation period whiles the highest (33.17) was from the 7-days duration. The low iodine value in the 5-day fermentation period may be due to earlier onset of oxidation which could have resulted in a decline in unsaturation. On the other hand, the high iodine value may also be due to a decline in oxidation resulting in high number of unsaturated bonds.

As regards the interaction between different drying methods and fermentation durations 5days fermentation and solar drying had the least IV. This may be due to the earlier onset of oxidation.

Oils have been classified into 3 groups based on iodine value; oil is classified as nondrying if the IV is less than 100; as semi-drying if between 100 and 130; or as drying if between 130 and 200 (Cocks *et al.,* 1966). The iodine values obtained in this study (23.82-36.00) was found to be lower than the specified range (80-106) by Food and Agricultural Organisation (FAO)/World Health Organisation(WHO), (2009) for edible vegetable oils and that reported by Ellis *et al.,* (2007) for *Treculia africana (*35.66). Thus, it would not be suitable for the production of industrial products such as paints, vanishes and surface coatings.

5.3 pH OF COCOA BEAN OIL

The lowest pH (4.39) for the fermentation duration was observed in the 7-day fermentation period whereas the highest (4.43) was from the 5-day duration.

With the different drying methods, the lowest pH (4.39) was recorded in the solar drying method whilst the highest (4.43) was also recorded in the sun drying method.

As regards the interaction between the different drying methods and fermentation durations, 7-day fermentation and sun drying had the least pH (4.33) whilst 7-day fermentation and solar drying recorded the highest pH (4.46). The values obtained in this study were within the pH range obtained in bread fruits by sun drying (4.32 -5.90) by Ellis *et al.,* (2007).

5.4 REFRACTIVE INDEX OF COCOA BEAN OIL

A refractive index of 68.53 was recorded for oil produced from cocoa beans that were solar dried and a refractive index of 68.43 was also recorded for oil produced from cocoa beans that were sun dried with no significant difference between them. This shows that the method of drying had no effect on the level of unsaturation of the oil and hence the refractive index of cocoa bean oil.

The 5-day fermentation duration recorded a refractive index of 68.40 as against a refractive index of 68.56 recorded by the 7-day fermentation period. There was no significant difference between the refractive indices of the 5-day and 7-day fermentation periods. This also implies that the duration of fermentation had no effect on the level of unsaturation of the oil.

Concerning the interaction between different drying methods and fermentation durations the refractive index values obtained were in the range of (68.30-68.56) with no significant difference between them. This shows that the interaction between different drying methods

and duration of fermentation had no effect on the level of unsaturation of the oil and hence the refractive index of the oil. Refractive index is directly proportional to the level of unsaturation. Hence, refractive index reduces linearly as iodine value also reduces. From this study, the range of refractive index values (68.30-68.56) recorded is higher than the range reported of some fats in the nut family (1.45-1.49) (Eckey, 1954). Again, it is also higher than the refractive index value of 1.47 obtained for *Treculia africana* seed oil. Comparing the range refractive index values obtained to that of *Treculia africana* seed oil and the range of values of some fats in the nut family shows that the level of unsaturation in the oil is high. The high refractive index value may be due to a decline in oxidation resulting in high number of unsaturated bonds (Nielson, 1994).

5.5 SAPONIFICATION VALUE OF COCOA BEAN OIL

Saponification value of 2.76 was recorded for oil produced from cocoa beans that were solar dried and a refractive index of 2.79 was also recorded for oil produced from cocoa beans that were sun dried with no significant difference between them. This shows that the method of drying had no effect on the average molecular weight of all triacylglycerols of the oil.

The 5-day fermentation duration recorded a saponification value of 2.97 as against a saponification value of 2.57 recorded by the 7-day fermentation period. There was no significant difference between the refractive indices of the 5-day and 7-day fermentation periods. This also implies that the duration of fermentation had no effect on the average molecular weight of all triacylglycerols of the oil and hence had no effect also on the saponification value of the oil.

Concerning the interaction between different drying methods and fermentation durations the saponification values obtained were in the range of (2.23-3.35) with no significant difference between them. This shows that the interaction between different drying methods and duration of fermentation had no effect on the average molecular weight of all triacylglycerols of the oil and hence no effect also on the saponification value of the oil.

The values obtained in this study were quite lower than that reported on palm kernel oil (247.0 mg/KOH/g), Jatropha curcas oil (202.6 mg/KOH/g) and palm oil (201.0 mg/KOH/g) by Ankapong, (2010). The saponification values obtained in this study were also lower than the (128.33) reported on *Treculia africana* seeds by Ellis *et al.,* (2007).

The results obtained in this study suggest that the oils obtained might not useful in the liquid soap and shampoo production industries.

5.6 FREE FATTY ACID CONTENT OF COCOA BEAN OIL

A free fatty acid (FFA) content of 1.39% was recorded for oil produced from cocoa beans that were solar dried and a free fatty acid content of 1.24% was also recorded for oil produced from cocoa beans that were that were sun dried with no significant difference between them. This shows that the method of drying had no effect on the percentage of fatty acids liberated from the triglyceride chain of the oil.

The lowest FFA content 1.02% for fermentation duration was recorded for oil produced from the 5-day period whereas the highest FFA content of 1.63% was also recorded for the 7-day fermentation period. The high FFA content in the 7-day fermentation period could be attributed to increased biochemical activities during the 7-day fermentation process.

With regards to interaction between different methods of drying and different fermentation durations, 5-day solar drying recorded the lowest FFA content 0.92% whilst 7-day solar drying recorded the highest free fatty acid content (1.88). This may be due to the high microbial (biochemical) activity with increased fermentation period which increases the free fatty acid contents liberated from the triglyceride chain in the cocoa bean oil.

The free fatty acids obtained in this study for the different fermentation duration were found to be higher than 0.2 to 0.8% reported on whole healthy cocoa beans from Côte d'Ivoire by Guehi *et al.,* (2008), however, the findings from this study were quite close to the UE standards (1.75% oleic acid equivalent).

5.7 RELATIVE DENSITY OF COCOA BEAN OIL

Cocoa bean oil produced from beans that were solar dried or sun dried recorded a relative density of 0.88 and 0.86 respectively, with no significant difference between them.

The 5-day and 7-day fermentation durations all recorded a relative density of 0.87. Concerning the interaction between different drying methods and fermentation durations, the relative density values obtained were in the range of (0.86-0.88) with no significant difference between them. This shows that the interaction between different drying methods and duration of fermentation had no effect on the relative density of the oil.

The density of vegetable oil is lower than that of water and the differences between vegetable oils are quite small, particularly among the common vegetable oils. Generally, the density of oil decreases with molecular weight, but increase with unsaturation level (Gunstone, 2004).

From this study, the range of relative density values (0.86-0.888) recorded are lower than the range reported of moringa oleifera seed samples (0.95-1.05) (Adejumo *et al.,* 2013).

5.8 PEROXIDE VALUE OF COCOA BEAN OIL

Peroxide value of 21.33 was recorded for both oils produced from cocoa beans that were either solar dried or sun dried. The mean peroxide values for the different drying methods were quite higher than (6.14) and (6.02) reported on palm oil at the Mallam Atta and the 31st December market respectively. The mean peroxide value (PV) of *Treculia africana* seed oil reported by Ellis *et al.,* (2007), was (2.67) and was quite lower than that reported in this research.

The 5-day fermentation duration recorded a peroxide value of 23.33 and a peroxide value of 19.33 recorded by the 7-day fermentation period. There was no significant difference between the peroxide values of the 5-day and 7-day fermentation periods. This also implies that the duration of fermentation had no significant effect on the peroxide value of the oil.

As regards the interaction between different drying methods and fermentation durations 7-day fermentation and solar drying had the least peroxide (17.33) value whilst 5-day fermentation duration and solar drying recorded the highest peroxide value (25.33).

The high values of peroxide recorded could be due to high temperatures due to heat buildup with the commencement of the process of fermentation (Tomlins *et al.,* 1993).

This was not surprising as the least fermentation duration (5-days) had the highest (23.33) peroxide value. On the seventh day of fermentation, temperature in the heap of cocoa reduces due to ceasing metabolic activities resulting in low oxidation. This might have

contributed to the low perioxide value recorded for the seven day compared to the fifth day.

The peroxide value is usually used as an indicator of deterioration by rancidity of fats or oils. It can be observed that the oils produced from both sun and solar drying methods all showed signs of rancidity as per their peroxide values. This is because the peroxide value from the study is above the range (0-10) for fresh fats and oils (Kirk and Sawyer, 1991). Again the peroxide values obtained falls outside the Codex Alimentarious Standard (PV < 10 Eq/kg fat or oil) for oils which are edible. This results suggests that the different drying methods all resulted in high perioxide values of oil. This may be due to the low saturated fatty acids in cocoa which enhance rancidity (Baidoo, and Johnson, 2002). When peroxide values are > 20, it signifies that the fats or oils are of very poor quality with substantial off flavours (Baidoo, and Johnson, 2002).

5.9 TOTAL PHENOLIC CONTENT OF COCOA BEAN OIL

The lowest phenolic content (68.60) was recorded for the oil produced from sun dried cocoa beans whereas the highest phenolic content (1918.20) was also recorded for the oil produced from solar dried cocoa beans.

The lowest phenol content (607.6) for the fermentation duration was observed in the 7days fermentation period whiles the highest (1379.2.) was from the 5-days fermentation duration.

As regards the interaction between different drying methods and fermentation durations 5-days fermentation and sun drying had the least Phenolic content (68.1), followed by 7days fermentation and sun drying (69.1), with no significant difference between them.
The highest phenol content was observed in the 5-days fermentation and solar drying.

Phenols are antioxidant agents which terminates free radicals and also takes part in retarding degradation of lipids through oxidation (Pourmorad *et al.,* 2006). Hence, its presence in cocoa bean oil suggests value addition in the areas of nutrition and health.

5.10 YIELD OF COCOA BEAN OIL

From this study, the method of drying had no effect on the percentage of oil yield in the cocoa beans. The 5-day fermentation duration recorded an oil yield of 24.10% as against an oil yield of 28% recorded by the 7-day fermentation period. There was no significant difference between the oil yields of the 5-day and 7-day fermentation periods.

As regards the interaction between different drying methods and fermentation durations, 7-days fermentation and sun drying had the highest oil yield (28.20%) whilst 5-day fermentation and solar drying had the least yield. The range of values from this study is comparatively lower than the range of oil yield (32-40%) reported in other parts of the world (Lalas and Tsaknis, 2002).

Based on the comparatively low oil yield of cocoa beans, it suggests that cocoa beans (seeds) cannot be used as a replacement for local seeds which are rich in oil. This is because; it will not be economically feasible.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The effect of different fermentation durations and different drying methods and their interactive effect on the quality of cocoa bean oil have been studied and conclusions can be made from the outcome of this investigation.

The study revealed that, though the cocoa bean oil produced from both 5-day and 7-day fermentation durations all had acid values and free fatty acid percentages with significant differences between them but they were all within the range for consumption (0-10). On the other hand, oil produced from 7-day fermentation duration of cocoa beans had the least peroxide value which is below 20. This PV value gives it a comparative advantage over the others, as when peroxide values are > 20, it signifies that the fats or oils are of very poor quality with substantial off flavours. Therefore, conclusion could be drawn that the 7-day fermentation period produced the best quality oil among two different fermentation durations.

On the different drying methods, both solar and sun drying all had the same peroxide value which was above 20. Again, though the acid values and free fatty acid contents of the oils produced from the different drying methods were numerically different with sun drying having a slight advantage over solar drying, but they were not significantly different. Furthermore, the acid values and free fatty acid contents were all within the acceptable range for consumption. It could therefore be concluded that all the different methods of drying produced good oil but sun drying produced an insignificantly better oil than solar drying.

From the results of interactive effect of the different drying methods and fermentation periods on the quality of cocoa bean oil, it could be concluded that the 7-day fermentation duration and solar drying produced the best quality oil among all the interactive effects.

It was observed that the yield of oil obtained ranged from (24.10 -29.00%). The peroxide value obtained was so high (19.33 -25.33%) compared to acceptable fresh oil (less than 10). The free fatty acid was within the acceptable range for consumption (0-10%). It was observed that 7 day fermentation with solar drying produced the best quality of oil since it had the least peroxide value (17.33) and high free fatty acid value (1.88). It was also observed that the 7 day fermentation resulted in a comparatively high oil quality than the 5 day fermentation.

6.2 RECOMMENDATION

- Further studies should be conducted on the oxidative stability and/or thermal stability of this oil to help estimate its shelf life.
- Blends of oil with other oils containing high antioxidants should also be investigated.

The lipid profile of oil extracted by the best method should be investigated.

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APPENDICES

APPENDIX A: ANALYSIS OF VARIANCE

Analysis of Variance Table for AV

Source	DF	SS	MS	F	P
DRY	1	0.6627	0.66270	2.26	0.1712 FER
1	6.3075	6.30750	21.51	0.0017	
DRY*FER	1	1.3736	1.37363	4.68	0.0624
Error	8	2.3459	0.29323		
Total	11	10.6897			

Grand Mean 2.7150 CV 19.95

Analysis of Variance Table for COLOUR

Source	DF	SS	MS	F	P
DRY	1	141.453	141.453	515.94	0.0000
FER	1	243.000	243.000	886.32	0.0000
DRY*FER	1	187.230	187.230	682.91	0.0000
Error	8	2.193	0.274		
Total	11	573.877			

Grand Mean 8.3833 CV 6.25

Analysis of Variance Table for IV

Source	DF	SS	MS	F	P
DRY	1	49.444	49.444	33.03	0.0004
FER	1	262.170	262.170	175.13	0.0000
DRY*FER	1	7.811	7.811	5.22	0.0517
Error	8	11.976	1.497		
Total	11	331.402			

Grand Mean 28.496 CV 4.29

Analysis of Variance Table for PH

Source	DF	SS	MS	F	P
DRY	1	0.00608	0.00608	60.75	0.0001
FER	1	0.00368	0.00368	36.75	0.0003
DRY*FER	1	0.02168	0.02168	216.75	0.0000
Error	8	0.00080	0.00010		
Total	11	0.03222			

Grand Mean 4.4125 CV 0.23

Analysis of Variance Table for RI

Source	DF	SS	MS	F	P
DRY	1	0.03000	0.03000	2.57	0.1475
FER	1	0.08333	0.08333	7.14	0.0282
DRY*FER	1	0.03000	0.03000	2.57	0.1475
Error	8	0.09333	0.01167		
Total	11	0.23667			

Grand Mean 68.483 CV 0.16

Analysis of Variance Table for SP

Source	DF	SS	MS	F	P
DRY	1	0.00301	0.00301	0.02	0.9030
FER	1	0.48401	0.48401	2.54	0.1494
DRY*FER	1	1.56241	1.56241	8.21	0.0210
Error	8	1.52227	0.19028		
Total	11	3.57169			

Grand Mean 2.7758 CV 15.71

Analysis of Variance Table for FFA

Source	DF	SS	MS	F	P
DRY	1	0.06750	0.06750	0.85	0.3832 FER
1	1.10413	1.10413	13.92	0.0058	
DRY*FER	1	0.38163	0.38163	4.81	0.0596
Error	8	0.63440	0.07930		
Total	11	2.18767			

Grand Mean 1.3233 CV 21.28

Analysis of Variance Table for RD

Source	DF	SS	MS	F	P
DRY	1	0.00068	6.750E-04	6.75	0.0317
FER	1	0.00008	7.500E-05	0.75	0.4117
DRY*FER	1	0.00008	7.500E-05	0.75	0.4117
Error	8	0.00080	1.000E-04		
Total	11	0.00163			

Grand Mean 0.8725 CV 1.15

Analysis of Variance Table for PV

Source	DF	SS	MS	F	P
DRY	1	1.950E-30	1.950E-30	0.00	1.0000
FER	1	48.0000	48.0000	9.00	0.0171
DRY*FER	1	48.0000	48.0000	9.00	0.0171
Error	8	42.6667	5.33333		
Total	11	138.667			

Grand Mean 21.333 CV 10.83

Analysis of Variance Table for TP

Source	DF	SS	MS	F	P
DRY	1	1.026E+07	1.026E+07	6665.23	0.0000
FER	1	1786269	1786269	1160.06	0.0000
DRY*FER	1	1790902	1790902	1163.07	0.0000
Error	8	12318.5	1539.81		
Total	11	1.385E+07			

Grand Mean 993.38 CV 3.95